1118-Pos Board B28
A structural Role for Serotonin Transporter Terminal Domains in Uptake Regulation
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Serotonin transporter (SERT) is a member of the Na^+/Cl^--dependent neurotransmitter transporter family (NSS or SLC6), which also includes norepinephrine transporter (NET) and dopamine transporter (DAT). SERT mediates rapid removal and recycling of released serotonin following neuronal stimulation. The activity of SERT is tightly regulated, as its misfunction is associated with diseases such as autism, bipolar disorder, depression, obsessive-compulsive disorder, migraine and anxiety. Regulation is modulated by post-translational modifications, including phosphorylation, glycosylation as well as by interaction with other proteins. The majority of this regulatory behavior involves the N- and/or C-terminal domains of the protein, the structure of which is not known. In order to determine the structural determinants of the SERT regulation we have generated a model of full-length SERT using a combination of template-based and *de Novo* modeling methodologies. The modeling process was carried out in three stages: a) modeling of the transmembrane segment of SERT using the X-ray structure of the bacterial homolog LeuT, b) modeling of both N- and C-terminal domains of the protein using a *de Novo* folding approach (Rosetta) and c) modeling of the full complex. The last two steps required extensive conformational sampling to ensure that native-like states were modeled. The best models were selected out using clustering and energetic criteria. We present a comparison of the models to the available experimental data.

1119-Pos Board B29
The Human Transporter Associated with Antigen Processing (TAP): A Computational Study Focused on the Nucleotide Binding Domains
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The human Transporter Associated with Antigen Processing (TAP) is the only member of the ATP-Binding Cassette (ABC) transporter family with a crucial role in the adaptive immune response, as it is involved in the antigen presentation pathway. TAP resides in the entoplasmic reticulum (ER) membrane, and shuttles peptides derived from proteasomal degradation into the major histocompatibility complex (MHC) class I molecules, for their further presentation at the cell surface. TAP is a heterodimer formed by two subunits, TAP1 and TAP2, organized in four main domains: two N-terminal transmembrane domains (TMDs), defining the translocation pathway, and two highly conserved C-terminal nucleotide binding domains (NBDs). The NBD dimerization driven by ATP binding and hydrolysis is the key step of the catalytic cycle: this reversible association/dissociation is directly coupled with the conformational changes occurring in the TMDs, so that peptides bound to the cytosolic side of the transporter can be released into the ER lumen.
The two ATP binding sites in TAP are not equivalent, and specific amino acid differences between them are responsible for a highly conserved binding site (consensus site) in TAP2, and a degenerate binding site in TAP1. This NBD asymmetry is the topic of the present study. In particular, we use computer simulations to describe the different nucleotide binding properties of the two NBDs in TAP. Homology models of TAP-NBDs were built using the crystal structure of the bacterial transporter Sav1866 as a template, and different nucleotide bound states were generated: ADP1-ADP2, ATP1-ADP2, ADP1-ATP2, ATP1-ATP2. Molecular dynamics simulations were then used as a tool to investigate the dynamics of these heterodimers, focusing on the mechanism of the NBD opening as a function of the nucleotide-bound state.

1120-Pos Board B30
Molecular Simulations Distinguish Rhodopsin Counterion Models by Retinal Polyene Fluctuations
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We investigated retinal conformational changes within the binding cavity of rhodopsin through all-atom molecular dynamics (MD) simulations on the microsecond time scale. Two distinct pathways involving post-isomerization release of retinal conformational strain were tested. The MD trajectories for the counterion-switch and complex-counterion mechanisms [1] show that retinal experiences completely distinct geometrical rearrangements, yielding differences in its orientation and conformation. In the counterion-switch simulation the dihedral angle C11=C12-C13=C14 fluctuates, and is correlated with changes in the C5-, C9-, and C13-methyl group orientations with respect to the membrane normal. For the complex-counterion simulation, changes in the dihedral angle C7=C8-C9=C10 allow the methyl group orientation to remain relatively unaffected (~60° for the C5-, C9-, and C13-methyl groups), consistent with ^{2}H NMR data [2,3]. The C5=C6-C7=C8 and C9=C10-C11=C12 torsion angles also experience significant fluctuations, but at different times in each counterion simulation and not in conjunction with retinal methyl reorientation. In addition to retinal polyene chain analysis, the calculated ^{2}H NMR spectra from each simulation clearly demonstrate that the retinal methyl orientations for the complex-counterion mechanism accurately reproduce experimental ^{2}H NMR data for Meta I [2,3], whereas the counterion simulation does not. Rhodopsin simulations on the microsecond time scale are essential for modeling rhodopsin activation up to the Meta I photointermediate, because retinal polyene chain fluctuations occur as late as ~1500 ns into each simulation. This work builds on our previous computational studies examining rhodopsin in the dark and Meta I states [1,4] and provides valuable insights into the rhodopsin activation process. [1] K. Martínez-Mayorga *et al.* (2006) *JACS***128**, 16502-16503. [2] G.F.J. Salgado *et al.* (2006) *JACS***128**, 11067-11071. [3] A.V. Struts et al. (2007) *JMB***372**, 50-66. [4] P.-W. Lau *et al.* (2007) *JMB***372**, 906-917.

1121-Pos Board B31
Theoretical Investigation of Interactions between the Truncated Hemoglobin N from Mycobacterium Tuberculosis and Biological Membranes
Julie-Anne Rousseau, Michel Guertin, Patrick Lagüe.
The truncated hemoglobin HbN (trHbN) from the pathogenic bacterium *Mycobacterium tuberculosis* has a potent ability to detoxify •NO to nitrate (nitric oxide dioxygenase (NOD reaction)) and to protect aerobic respiration from inhibition by •NO in stationary phase cells of M. bovis BCG. HbN catalyses the rapid oxidation of •NO to innocuous nitrate (HbN-FeII(O2) + •NO –> HbN-FeIII + NO3-), with a second-order rate constant k′NOD approximately 745 $\mu M^{-1}\ s^{-1}$ (23 °C). This protein is thought to play pivotal roles in the cellular metabolism of the bacterium during stress and hypoxia and thus in the persistence of mycobacterial infection. Crystallographic studies and molecular dynamics (MD) simulations revealed that trHbN contains hydrophobic cavities forming four tunnels termed ST, LT, EHT and BET tunnels connecting the active site to the solvant. Recent studies have confirmed that apolar ligands use the tunnels to reach the buried active site from the bulk solvent (Daigle et al., submitted).
Until now, trHbN characterization has been done in solution despite the fact that the apolar substrates are more soluble (~ 3-fold) in biological membranes than in bulk solvent. In this study, we present the results obtained from molecular modelling tools of the interactions of trHbN with model membranes. Preliminary results using implicit solvent suggest that trHbN inserts into the membrane so that the ST and EHT openings are located in the center of the membrane, where the concentration of apolar substrates is the highest and that LT opening is located at the membrane interface. We propose that such positioning may be related to the role of the protein. More results from all-atoms MD simulations will be presented.

1122-Pos Board B32
Computational Prediction of Cellulose Synthase Protein Structure
Andrés Vargas, Latsavongsakda Sethaphong, Yaroslava Yingling.
Cellose synthases (CesAs) are the proteins responsible for the polymerization of cellulose from glucose. They are transmembrane proteins that take their substrate from within the cytosol of the cell and extrude the product to the exterior. Due to the fact that they are found on the membrane and that it is difficult to crystallize such proteins, the structure of CesAs has not been determined. However, the ability of CesAs to form rather large complexes with each other has been observed under optical microscopy. CesAs can form protein rosettes from three slightly different CesA genes. One of these genes bears a region similar to the binding site of glycosyltransferase genes. We used ab-initio protein structure prediction and molecular dynamics to predict catalytic domain of cotton CesA. The globular catalytic region from this gene, which lies in the cytosol, revealed to contain a stretch of beta sheets flanked by a UDP-glucose binding site. Moreover, we predicted the overall position of CesA towards the membrane that may be linked to the extrusion of the cellulose from the catalytic region to the exterior of the cell.